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Preparation of 177Lu-DOTA-Trastuzumab: an insight into the in-house optimized radiochemistry procedures employed for patient dose preparation

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Objective: Trastuzumab (Herceptin®), a humanized monoclonal antibody, is an approved agent used for immunotherapeutic treatment of metastatic breast cancer as it targets HER2 (human epidermal growth receptors 2) receptors over-expressed in such cancer cells. Therefore, radiolabeled Trastuzumab is expected to have significant potential as a radioimmunotherapeutic agent for the treatment of ca breast patients over-expressing HER2 receptors. The aim of the present study is to standardize the formulation protocol of ^{177}Lu-Trastuzumab and scale-up the preparation for administration in patients.

Methodology: Trastuzumab was conjugated with a suitable bi-functional chelating agent (BFCA) namely, p-NCS-benzyl-DOTA by incubating Trastuzumab (5 mg, 35 nmol) and p-NCS-benzyl-DOTA (190 µg, 350 nmol) in sodium carbonate buffer (pH=9.5, 0.2 M) at 37 ℃ for 17 h. Post-incubation, the reaction mixture was purified using Amicon ultra-centrifugal units (MW cut off 10kDa) using NaOAc buffer (pH=5.0, 0.2 M). Determination of average number of p-NCS-benzyl-DOTA molecules attached per antibody moiety was carried out by UV-Vis spectrophotometry as well as by mass spectrometry using MALDI-TOF technique. ^{177}Lu-Trastuzumab complex was prepared by incubating the purified Trastuzumab-BFCA conjugate (2 mg, 13 nmol) with $^{177}LuCl_3$ [150 µL, 80 mCi (2.96 GBq)] at 37 ℃ for 90 min at pH ˜5.5. Percentage radiolabeling yield (%RCY) of the radiolabeled formulation was determined by paper chromatography (PC) using 0.1M sodium citrate solution as the mobile phase and high performance liquid chromatography (HPLC) using 0.05 M phosphate buffer with 0.05% sodium azide as the mobile phase. The radiolabeled preparation was purified by PD10 desalting columns using 0.2 M NaOAc buffer as the eluting solvent. The stability of the purified ^{177}Lu-Trastuzumab complex was determined till 4 days post-preparation by incubating the complex in phosphate buffered saline (PBS) at room temperature and carrying out the quality control analyses following the procedures mentioned above at various time intervals. The purified ^{177}Lu-Trastuzumab formulation was administered in 08 ca breast patients [˜5 mCi (185 MBq) in each patient] for studying preliminary pharmacokinetics and biological distribution of the agent.

Results and Discussion: An average of 7.5±1.2 p-NCS-benzyl-DOTA molecules were found to be attached per Trastuzumab moiety. HPLC studies showed that the ^{177}Lu-Trastuzumab conjugate could be prepared with a %RCY of 75.78±3.56 (Rt = 15.5 min and 21.5 min for ^{177}Lu-Trastuzumab and free $^{177}LuCl_3$, respectively), which was subsequently improved to >95 by purification through PD10 column (with average recovery of 72.8±1.2%). In-vitro stability studies showed that the %RCY of ^{177}Lu-Trastuzumab decreased to 84.15±1.57 after 4 days of storage at room temperature in PBS. Clinical studies in ca breast patients revealed the accumulation of the radiolabeled antibody at breast cancer lesions with slow but gradual clearance of activity from blood and other non-target organs.

Conclusion: An in-house procedure for the formulation of patient dose of ^{177}Lu-Trastuzumab was optimized. Preliminary clinical imaging studies revealed the retention of affinity of Trastuzumab towards the disease after functional modifications and radiolabeling procedures.

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